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(54) Title: TRANSCRIPTIONAL CO-REPRESSOR THAT INTERACTS WITH NUCLEAR HORMONE RECEPTORS AND USES THEREFOR

(57) Abstract

In accordance with the present invention, there are provided novel receptor interacting factors, referred to herein as "SMRT", i.e., a Silencing Mediator (co-repressor) for Retionic Acid Receptor (RAR) and Thyroid hormone Receptor (TR). SMRT is anovel protein whose association with RAR and TR both in solution and on DNA response elements is destabilized by ligand. The interaction of SMRT with mutant receptors correlates with the transcriptional silencing activites of receptors. *in vivo*, SMRT functions as a potent co-repressor. A GAL4 DNA binding domain (DBD) fusion of SMRT behaves as a frank repressor of a GAL4-dependent reporter. Together, these data identify a novel class of cofactor which is believed to represent an important mediator of hormone action.

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Transcriptional Co-repressor that Interacts with Nuclear Hormone Receptors and Uses Therefor

FIELD OF THE INVENTION

The present invention relates to intracellular receptors, methods for the modulation thereof, and methods for the identification of novel ligands therefor. In a particular aspect, the present invention relates to methods for the identification of compounds which function as ligands (or ligand precursors) for intracellular receptors. In another aspect, the present invention relates to novel chimeric constructs and uses therefor.

BACKGROUND OF THE INVENTION

A central problem in eukaryotic molecular biology continues to be the elucidation of molecules and mechanisms that mediate specific gene regulation. As part of the scientific attack on this problem, a great deal of work has been done in efforts to identify ligands (i.e., exogenous inducers) which are capable of mediating specific gene regulation. Additional work has been done in efforts to identify other molecules involved in specific gene regulation.

Although much remains to be learned about the specifics of gene regulation, it is known that ligands modulate gene transcription by acting in concert with intracellular components, including intracellular receptors and discrete DNA sequences known as hormone response elements (HREs).

The identification of compounds which directly or indirectly interact with intracellular receptors, and thereby affect transcription of hormone-responsive genes,

would be of significant value, e.g., for therapeutic applications.

Transcriptional silencing mediated by nuclear receptors plays an important role in development, cell differentiation, and is directly linked to the oncogenic activity of v-erbA. The mechanism underlying this effect is unknown but is one key to understanding the molecular basis of hormone action. Accordingly, the identification of components involved in transcriptional silencing would represent a great advance in current understanding of mechanisms that mediate specific gene regulation.

Other information helpful in the understanding and practice of the present invention can be found in commonly assigned United States Patent Nos. 5,071,773 and 4,981,784; and United States Patent Application Nos. 325,240, filed March 17, 1989; 370,407, filed June 22, 1989; and 438,757, filed November 16, 1989, all of which are hereby incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have discovered a novel receptor interacting factor, referred to herein as "SMRT", i.e., a silencing mediator (co-repressor) for retinoic acid receptor (RAR) and thyroid hormone receptor (TR). SMRT is a novel protein whose association with RAR and TR both in solution and on DNA response elements is destabilized by ligand. The interaction of SMRT with mutant receptors correlates with the transcriptional silencing activities of receptors.

In vivo, SMRT functions as a potent co-repressor. A GAL4 DNA binding domain (DBD) fusion of SMRT behaves as a frank repressor of a GAL4-dependent reporter. Together,

these data identify a novel class of cofactor which is believed to represent an important mediator of hormone action.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the quantitation by phosphoimager of a dose-dependent dissociation of SMRT from RAR or TR by all-*trans* retinoic acid (atRA) or thyroid hormone (triiodothyronine or T3).

Figure 2 presents amino acid (aa) sequences of SMRT (Genbank accession number XXXXX). The aa sequence presented in parentheses (i.e., residues 1330-1376) is an alternatively spliced insert which is not present in the original two-hybrid clone (C-SMRT, aa 981 to C-terminal end). The proline-rich N-terminal domain (aa 1-160) and the glutamine-rich region (aa 1061-1132), as well as the ERDR and SG regions, are also indicated. The C-terminal region of SMRT (aa 1201 to C-terminal end) shows 48% aa identity to RIP13 (Seol et al., *Molecular Endocrinology* **9**:72-85 (1995)). The rest of the sequence of RIP13 shows 22% aa identity to SMRT (aa 819-1200).

Figure 3 illustrates mediation of the silencing effect of hRARα and hTRβ by SMRT *in vivo*.

Figure 3(A) illustrates that v-erbA reverses the silencing effect of GAL-RAR (GAL4 DBD-hRARα 156-462) while SMRT restores the silencing effect.

Figure 3(B) illustrates that the RAR403 truncation mutant reverses the silencing effect of GAL-TR (GAL4 DBD-hTRβ 173-456) while SMRT restores the silencing effect.

Figure 3(C) illustrates that v-erbA and full length SMRT or C-SMRT have no effect on GAL-VP16 activity.

Figure 3(D) illustrates that a GAL4 DBD fusion of full length SMRT suppresses the thymidine kinase basal promoter activity containing four GAL4 binding sites. The fold of repression was calculated by dividing the normalized luciferase activity transfected with the GAL4 DBD alone by those transfected with indicated amount of GAL DBD fusion constructs.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided co-suppressors of steroid/thyroid hormone receptor activity, said co-suppressors having a structure and function characteristic of the silencing mediator for retinoic acid receptor and thyroid hormone receptor.

Co-suppressors contemplated by the present invention have substantially the same sequence as residues 1-1329 plus 1376-1495, as set forth in SEQ ID NO:1, optionally further comprising the amino acid residues set forth in SEQ ID NO:2 (i. e., residues 1330-1375 of SEQ ID NO:1).

The phrase "substantially the same" is used herein in reference to the nucleotide sequence of DNA, the ribonucleotide sequence of RNA, or the amino acid sequence of protein, that have slight and non-consequential sequence variations from the actual sequences disclosed herein. Species that are substantially the same are considered to be equivalent to the disclosed sequences and as such are within the scope of the appended claims. In this regard, "slight and non-consequential sequence variations" mean that sequences that are substantially the same as the DNA, RNA, or proteins disclosed and claimed herein are

functionally equivalent to the sequences disclosed and claimed herein. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein. In particular, functionally equivalent DNAs encode proteins that are the same as those disclosed herein or that have conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue. These changes include those recognized by those of skill in the art as those that do not substantially alter the tertiary structure of the protein.

In accordance with another aspect of the present invention, there are provided antibodies raised against the above-described co-suppressor. Such antibodies can be employed for studying tissue localization of invention co-repressor, the structure of functional domains, the purification of receptors, as well as in diagnostic applications, therapeutic applications, and the like. Preferably, for therapeutic applications, the antibodies employed will be monoclonal antibodies.

The above-described antibodies can be prepared employing standard techniques, as are well known to those of skill in the art, using the invention co-repressor or portions thereof as antigens for antibody production. Both anti-peptide and anti-fusion protein antibodies can be used [see, for example, Bahouth et al. (1991) Trends Pharmacol Sci. vol. 12:338-343; Current Protocols in Molecular Biology (Ausubel et al., eds.) John Wiley and Sons, New York (1989)]. Factors to consider in selecting portions of invention co-repressor for use as immunogen (as either a synthetic peptide or a recombinantly produced bacterial fusion protein) include antigenicity, accessibility (i.e., where the selected portion is derived from, e.g., the

ligand binding domain, DNA binding domain, dimerization domain, and the like), uniqueness of the particular portion selected (relative to known receptors and co-suppressors therefor), and the like.

In accordance with yet another aspect of the present invention, there are provided methods to block the repressing effect of invention co-suppressors, said method comprising administering an effective amount of an antibody as described herein. Alternatively, a silencing domain of a nuclear receptor can be employed. Those of skill in the art can readily determine suitable methods for administering said antibodies, and suitable quantities for administration, which will vary depending on numerous factors, such as the indication being treated, the condition of the subject, and the like.

In accordance with a still further aspect of the invention, there are provided isolated polynucleic acids encoding the above-described co-suppressor. In addition, there are also provided vectors containing the above-described polynucleic acid.

In accordance with a still further aspect of the present invention, there are provided complexes comprising the above-described co-suppressor and a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors, wherein said member contains a silencing domain which represses basal level promoter activity of target genes. Homodimeric or heterodimeric members of the steroid/thyroid hormone superfamily of receptors contemplated for use herein include thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, retinoic acid receptor-retinoid X receptor heterodimer, retinoid X receptor homodimer, and the like.

The above-described complexes optionally further comprise a response element for the member of the steroid/thyroid hormone superfamily of receptors. Such response elements are well known in the art. Thus, for example, RAR response elements are composed of at least one direct repeat of two or more half sites separated by a spacer of five nucleotides. The spacer nucleotides can independently be selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Similarly, TR response elements can be composed of the same half site repeats, with a spacer of four nucleotides. Alternatively, palindromic constructs as have been described in the art are also functional as TR response elements.

The above-described co-repressor/dimeric receptor complexes can be dissociated by contacting complex with a ligand for the member of the steroid/thyroid hormone superfamily of receptors.

As employed herein, the term "ligand (or ligand precursor) for a member of the steroid/thyroid hormone

superfamily of receptors" (i.e., intracellular receptor) refers to a substance or compound which, in its unmodified form (or after conversion to its "active" form), inside a cell, binds to receptor protein, thereby creating a ligand/receptor complex, which in turn can activate an appropriate hormone response element. A ligand therefore is a compound which acts to modulate gene transcription for a gene maintained under the control of a hormone response element, and includes compounds such as hormones, growth substances, non-hormone compounds that modulate growth, and the like. Ligands include steroid or steroid-like hormone, retinoids, thyroid hormones, pharmaceutically active compounds, and the like. Individual ligands may have the ability to bind to multiple receptors.

Accordingly, as employed herein, "putative ligand" (also referred to as "test compound") refers to compounds such as steroid or steroid-like hormones, pharmaceutically active compounds, and the like, which are suspected to have the ability to bind to the receptor of interest, and to modulate transcription of genes maintained under the control of response elements recognized by such receptor.

Examples of known ligands include all-*trans*-retinoic acid (ligand for retinoic acid receptor), 9-*cis*-retinoic acid (ligand for retinoid X receptor), thyroid hormone (ligand for thyroid hormone receptor), 1,25-dihydroxy vitamin D_3 (ligand for vitamin D_3 receptor), and the like.

In accordance with another aspect of the present invention, there is provided a method to repress the activity of a member of the steroid/thyroid hormone superfamily of receptors containing a silencing domain which represses basal level promoter activity of target genes, said method comprising contacting said member of the

steroid/thyroid hormone superfamily of receptors with a sufficient quantity of a co-suppressor as described hereinabove so as to repress the activity of said member. Members of the superfamily contemplated for repression in accordance with this aspect of the present invention include thyroid hormone receptor, retinoic acid receptor, vitamin D receptor, peroxisome proliferator activated receptor, and the like.

In accordance with yet another aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, said method comprising comparing the size of the above-described co-suppressor/dimeric receptor complex (i.e., complexes comprising the above-described co-suppressor and a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors) upon exposure to test compound, relative to the size of said complex in the absence of test compound. An observed size corresponding to intact complex is indicative of an inactive compound, while an observed size that reflects dissociation of the complex is indicative of a compound that disrupts the complex, thereby relieving the suppression caused thereby. Optionally, the complex employed in this assay further comprises a response element for said member of the steroid/thyroid hormone superfamily of receptors.

The size of the above-described complex can readily be determined employing various techniques available in the art. For example, electrophoretic mobility shift assays (EMSA) can be employed (wherein receptor alone or receptor-co-suppressor complex is bound to target DNA and the relative mobility thereof determined). Those of skill in the art can readily identify other methodology which can be employed to

determine the size of the complex as a result of exposure to putative ligand.

In accordance with a still further aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, without substantially activating said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

optionally, invention co-suppressor, and

wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains

hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

optionally, invention co-suppressor, and thereafter

selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, but substantially lacking the ability to activate steroid/thyroid hormone receptor activity.

The addition of invention co-suppressor is optional in the above-described assay because it is present endogenously in most host cells employed for such assays. It is preferred, to ensure the presence of a fairly constant amount of co-suppressor, and to ensure that co-suppressor is not a limiting reagent, that co-suppressor be supplied exogenously to the above-described assays.

Mutant receptors contemplated for use in the practice of the present invention are conveniently produced

by expression plasmids, introduced into the host cell by transfection. Mutant receptors contemplated for use herein include RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers, TR160-containing heterodimers, and the like.

Reporter constructs contemplated for use in the practice of the present invention comprise:

(a) a promoter that is operable in the host cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein the reporter protein-encoding DNA segment is operatively linked to the promoter for transcription of the DNA segment, and

wherein the hormone response element is operatively linked to the promoter for activation thereof.

Hormone response elements contemplated for use in the practice of the present invention are well known in the art, as has been noted previously.

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like. Exemplary promoters include the simian virus (SV) promoter or modified form thereof (e.g., ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)].

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented by the terms "GAL4 response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "GAL4 response element" was "turned on" or otherwise activated.

In practicing the above-described functional bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence and absence of a test compound to determine if the test compound is able to produce activation of the promoter operatively linked to the response element of the reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Any cell line can be used as a suitable "host" for the functional bioassay contemplated for use in the practice of the present invention. Thus, cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include Schneider cells, CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, yeast cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1

derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound. "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with yet another aspect of the present invention, there is provided a method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to relieve the suppression caused by a co-suppressor as described hereinabove, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of

receptors, wherein said response element is operatively linked to a reporter, and

optionally, invention co-suppressor, and

wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

optionally, invention co-suppressor, and thereafter

selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of compound, and

substantially the same reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

In accordance with a still further aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, and activate said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

optionally, invention co-suppressor, and

wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

optionally, invention co-suppressor, and thereafter

selecting those compounds which provide:

increased reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound, and

substantially increased reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor.

In accordance with still another embodiment of the present invention, there are provided modified forms of the above-described co-suppressor, including:

full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 DNA binding domain,

full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 activation domain,

full length silencing mediator for retinoic acid and thyroid receptors plus glutathione S-transferase (GST) tag,

and the like.

The above-described modified forms of invention co-suppressor can be used in a variety of ways, e.g., in the assays described herein.

An especially preferred modified co-suppressor of the invention comprises full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 activation domain.

In accordance with a still further embodiment of the present invention, there is provided a method to identify compounds which disrupt the ability of a co-suppressor as described hereinabove to complex with steroid/thyroid hormone receptors, without substantially activating said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor as described above,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

said modified co-suppressor,

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes, and

the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

a lower reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of disrupting the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with steroid/thyroid hormone receptors, without substantially activating said receptor.

Mutant receptors contemplated for use in this embodiment of the present invention include RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers, TR160-containing heterodimers, and the like.

Suitable host cells for use in this embodiment of the present invention include mammalian cells as well as yeast cells. Yeast cells are presently preferred because they introduce no background since SMRT (i.e., silencing mediator (co-repressor) for retinoic acid receptor (RAR) and thyroid hormone receptor (TR)) is not endogenous to yeast.

In accordance with yet another embodiment of the present invention, there is provided a method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor as described hereinabove, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor as described above,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises:

said modified co-suppressor,

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes, and

the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of compound, and

substantially the same reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of compound,

wherein said selected comounds are capable of activating steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt the complex of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors and a steroid/thyroid hormone receptor.

Suitable host cells for use in this embodiment of the present invention include mammalian cells as well as yeast cells. Yeast cells are presently preferred because

they introduce no background since SMRT is not endogenous to yeast.

In accordance with a still further embodiment of the present invention, there is provided a method to identify compounds which activate a steroid/thyroid hormone receptor, and disrupt the ability of a co-suppressor as described hereinabove to complex with said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor as described above,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

said modified co-suppressor,

the same homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member

is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes, and

the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

a reduction in reporter signal upon exposure of compound to said first expression system, relative to reporter signal in the absence of said compound, and

increased reporter signal upon exposure of compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of activating a steroid/thyroid hormone receptor and disrupting a complex comprising steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

Suitable host cells for use in this embodiment of the present invention include mammalian cells as well as yeast cells. Yeast cells are presently preferred because they introduce no background since SMRT is not endogenous to yeast.

In accordance with yet another aspect of the present invention, there is provided a method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor as described hereinabove to complex with said receptor, said method comprising:

comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

wherein said combination expression system comprises:

a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a second homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes (i.e., provides basal level expression),

wherein either said first homodimer (or heterodimer) or said second homodimer (or heterodimer) is operatively linked to a GAL4 DNA binding domain,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a first reporter,

a GAL4 response element, wherein said response element is operatively linked to a second reporter, and

optionally a co-suppressor of steroid/thyroid hormone receptor activity, said co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and thereafter

identifying as capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, but substantially lacking the ability to activate steroid/thyroid hormone receptor activity those compounds which provide:

a higher reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, or

identifying as capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and

substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

identifying as capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, and

a greater increase in reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound.

Thus, the change in expression level of the two different reporters introduced in a single transfection can be monitored simultaneously. Based on the results of this single transfection, one can readily identify the mode of interaction of test compound with the receptor/SMRT complex.

Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5'-CGGAGGACTGTCCTCCG-3' (SEQ ID NO:3),

such as, for example, 17MX, as described by Webster et al., in Cell 52:169-178 (1988), as well as derivatives thereof.

Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55:899-906 (1988); or Webster et al. in Cell 54:199-207 (1988).

In accordance with still another embodiment of the present invention, there is provided a method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor as described hereinabove to complex with said receptor, said method comprising:

comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

wherein said combination expression system comprises:

a modified co-suppressor as described above,

a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a second homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

wherein either said first homodimer (or heterodimer) or said

second homodimer (or heterodimer) is operatively linked to a GAL4 DNA binding domain,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a first reporter,

a GAL4 response element, wherein said response element is operatively linked to a second reporter, and thereafter

identifying as capable of disrupting the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with a steroid/thyroid hormone receptor, without substantially activating steroid/thyroid hormone receptor, those compounds which provide:

a lower reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, or

identifying as capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic

of the silencing mediator for retinoic acid and thyroid receptors, those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and

substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

identifying as capable of disrupting a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

a reduction in reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

increased reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound.

In accordance with a still further aspect of the present invention, there is provided a method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor as described hereinabove, said method comprising determining the effect of adding test compound to an expression system comprising:

a modified member of the steroid/thyroid hormone superfamily of receptors, wherein said modified member contains an activation domain which renders said receptor constitutively active,

a fusion protein comprising the receptor interaction domain of SMRT operatively linked to the GAL4 DNA binding domain, and

a GAL4 response element operatively linked to a reporter.

Prior to addition of an effective ligand for the member of the steroid/thyroid hormone superfamily of receptors employed herein, the association of the modified member and the fusion protein will be effective to bind the GAL4 response element and activate transcription of the reporter. The presence of an effective ligand is indicated by a reduction of reporter signal upon exposure to ligand, which disrupts the interaction of the modified member and fusion protein.

Activation domains contemplated for use in the practice of the present invention are well known in the art and can readily be identified by the artisan. Examples include the GAL4 activation domain, BP64, and the like.

To summarize, a novel nuclear receptor co-repressor which mediates the transcriptional silencing of RAR and TR has been identified. This discovery is of great interest because transcriptional silencing has been shown to play an important role in development, cell differentiation and the oncogenic activity of v-erbA (Baniahmad et al., *EMBO J.* **11**:1015-1023 (1992)); Gandrillon et al., *Cell* **49**:687-697 (1989)); Zenke et al., *Cell* **61**:1035-1049 (1990); Barlow et al., *EMBO J.* **13**:4241-4250 (1994); Levine and Manley, *Cell* **59**:405-408 (1989); Baniahmad et al., *Proc. Natl. Acad. Sci. USA* **89**:10633-10637 (1992b); and Saitou et al., *Nature* **374**:159-162 (1995)). In

fact, v-erbA mutants that harbor the Pro160->Arg change in the TR neither suppress basal transcription nor are capable of oncogenic transformation (Damm and Evans, (1993) supra).

The function of SMRT as a silencing mediator (co-repressor) of RAR and TR is analogous to mSin3 in the Mad-Max-Sin3 ternary complex (Schreiber-Agus et al., *Cell* **80**:777-786 (1995); and Ayer et al., *Cell* **80**:767-776 (1995)). Because GAL-SMRT functions as a potent repressor when bound to DNA, it is reasonable to speculate that the function of the unliganded receptors is to bring with them SMRT to the template via protein-protein interaction. Thus, the repressor function is intrinsic to SMRT as opposed to the TR or RAR itself (Baniahmad et al., *Proc. Natl. Acad. Sci. USA* **90**:8832-8836 (1993); and Fondell et al., *Genes Dev* **7**:1400-1410 (1993)). It is demonstrated herein that the ligand triggers a dissociation of SMRT from the receptor, which would lead to an initial step in the activation process. This would be followed (or be coincident) with an induced conformational change in the carboxy-terminal transactivation domain (τc, also called AF-2), allowing association with co-activators on the transcription machinery (Douarin et al., *EMBO J.* **14**:2020-2033 (1995); Halachmi et al., *Science* **264**:1455-1458 (1994); Lee et al., *Nature* **374**:91-94 (1995); and Cavailles et al., *Proc. Natl. Acad. Sci. USA* **91**:10009-10013 (1994)). Thus, as has previously been suggested (Damm and Evans (1993) supra), the ligand dependent activation of TR would represent two separable processes including relief of repression and net activation. The isolation of SMRT now provides a basis for dissecting the molecular basis of trans-repression.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1
Isolation of SMRT

Using a GAL4 DBD-RXR fusion protein (see, for example, USSN 08/177,740, incorporated by reference herein in its entirety) as a bait in a yeast two-hybrid screening system (Durfee et al. *Genes Dev* 7:555-569 (1993)), several cDNA clones encoding receptor interacting proteins were isolated. One of these proteins, SMRT, interacts strongly with unliganded RAR and TR but only weakly with RXR or other receptors in yeast. This protein was selected for further characterization.

Example 2
Far-western blotting procedure

Total bacteria extracts expressing GST fusions of hRARα (aa 156-462) or hRXRα LBD (aa 228-462) and control extracts expressing GST alone or GST-PML fusion protein were subjected to SDS/PAGE and electroblotted onto nitrocellulose in transfer buffer (25 mM Tris, pH 8.3/ 192 mM glycine/ 0.01% SDS). After denaturation/renaturation from 6 M to 0.187 M guanidine hydrochloride in HB buffer (25 mM Hepes, pH 7.7/25 mM NaCl/5 mM $MgCl_2$/1mM DTT) filters were saturated at 4°C in blocking buffer (5% milk, then 1% milk in HB buffer plus 0.05% NP40). *In vitro* translated ^{35}S-labeled proteins were diluted into H buffer (20 mM Hepes, pH 7.7/75 mM KCl/0.1 mM EDTA/2.5 mM $MgCl_2$/0.05% NP40/ 1% milk/1 mM DTT) and the filters were hybridized overnight at 4°C with (1 μM) or without ligand. After three washes with H buffer, filters were dried and exposed for autoradiography or quantitated by phosphoimager.

GST-SMRT is a GST fusion of the C-SMRT encoded by the yeast two hybrid clone. GST-SMRT has been purified, but contains several degradation products.

For yeast two-hybrid screening, a construct expressing the GAL4 DBD-hRXRα LBD (aa 198-462) fusion protein was used to screen a human lymphocyte cDNA library as described (Durfee et al., (1993) supra). Full length SMRT cDNA was isolated from a human HeLa cDNA library (Clontech) using the two-hybrid insert as a probe.

Using the above-described far-western blotting procedure, ^{35}S-labeled SMRT preferentially complexes with bacterial extracts expressing the RAR, marginally associates with RXR and shows no association with control extracts. In contrast, ^{35}S-PPAR selectively associates with its heterodimeric partner, RXR, but not with RAR. In a similar assay, ^{35}S-labeled RAR or TR interacts strongly with SMRT and their heterodimeric partner, RXR, but not with degraded GST products, while ^{35}S-RXR interacts only weakly with SMRT. Binding of ligand to RAR or TR reduces their interactions with SMRT but not with RXR, while binding of ligand to RXR has only slight effect. Figure 1 shows the quantitation of a dose-dependent dissociation of SMRT from RAR or TR by all-*trans* retinoic acid (atRA) or thyroid hormone (triiodothyronine or T3), demonstrating that the amount of ligand required for 50% dissociation in both cases are close to the kds for both ligands (Munoz et al. *EMBO J.* **7**:155-159 (1988); Sap et al., *Nature* **340**:242-244 (1989); and Yang et al., *Proc. Natl. Acad. Sci. USA* **88**:3559-3563 (1991)).

Full length SMRT encodes a polypeptide of 1495 amino acids rich in proline and serine residues (see Figure 2 and SEQ ID NO:1). Genbank database comparison reveals similarity of the C-terminal domain of SMRT to a partial cDNA encoding another receptor interacting protein, RIP13 (Seol et al., (1995) supra), whose role in receptor signaling is unknown. Within this region, there can be identified several potential heptad repeats which might mediate protein-protein interaction with the "a-helical

sandwich" structure (Bourguet et al., *Nature* **375**:377-382 (1995)) of the ligand binding domain (LBD) of receptors.

Example 3
Characterization of SMRT

Unlike other nuclear receptors, unliganded RAR and TR possess a strong silencing domain which represses basal level promoter activity of their target genes (Damm et al., *Nature* **339**:593-597 (1989); Brent et al., *New Biol.* **1**:329-336 (1989); Baniahmad et al., *Cell* **61**:505-514 (1990); and Baniahmad et al., *EMBO J.* **11**:1015-1023 (1992)). The preferential interaction of SMRT with RAR and TR in the absence of hormone suggests that SMRT may play a role in mediating the transcriptional silencing effect of the receptor.

To further investigate the involvement of SMRT in silencing, the interaction of SMRT with mutant receptors which display distinct silencing and/or transactivation activities was tested as follows. ^{35}S-methionine labeled receptors were used as probes to hybridize immobilized GST-SMRT in the presence (10 μM) or absence of all-*trans* retinoic acid (atRA). The total bacteria extract expressing GST-RXR was included as a control.

When quantitated by phosphoimager, RAR403 shows a 4-fold better interaction with SMRT than wild type RAR. Both full length RAR or a deletion mutant expressing only the ligand binding domain (LBD, referred to as $\Delta\Delta$R) associate with SMRT; this association is blocked by ligand.

These results confirm that the LBD alone is sufficient in the interaction. The carboxy-terminal deletion mutant RAR403 is a potent dominant negative suppressor of basal level promoter activity of RAR target genes (Damm et al., *Proc. Natl. Acad. Sci. USA* **90**:2989-2993

(1993); Tsai and Collins, *Proc. Natl. Acad. Sci. USA* **90**:7153-7157 (1993); and Tsai et al., *Genes Dev* **6**:2258-2269 (1992)). As might be predicted from the above studies, RAR403 and its amino terminal deletion derivative, ΔΔR403, interact strongly with SMRT in either the presence or absence of ligand, consistent with SMRT mediating the repressor activity of this mutant.

Example 4
Interaction of SMRT with TR Mutants

The interaction of SMRT with two different classes of TR mutants was analyzed next. The first mutant employed is the naturally occurring oncogene, v-erbA, which has strong silencing ability but no transactivation activity (Sap et al., (1989) supra; Sap et al., *Nature* **324**:635-640 (1986); Weinberger et al., *Nature* **318**:670-672 (1985); and Weinberger et al., *Nature* **324**:641-646 (1986)). The second mutant employed is a single amino acid change (Pro 160 -> Arg) of the rTRa (TR160) which has previously been shown to lose its capacity in basal level suppression but retains hormone dependent transactivation (Thompson et al., *Science* **237**:1610-1614 (1987); and Damm and Evans, *Proc. Natl. Acad. Sci. USA* **90**:10668-10672 (1993)). If SMRT is involved in silencing, it would be expected that SMRT should interact with the v-erbA, but show little or no association with the silencing-defective TR160 mutant.

Interaction of the oncogenic v-erbA and rTRα R160 mutant (TR160) with GST-SMRT was determined in a far-western assay as described above (see Example 2). When quantitated by phosphoimager, the v-erbA shows an 18-fold better interaction with SMRT than hTRβ, and the TR160 mutant shows a 10-fold lower signal than the rTRα.

As one might expect, v-erbA interacts strongly with SMRT both in presence or absence of ligand. In

contrast, full length TR160 mutant or LBD of TR160 (ΔΔTR160) does not interact significantly with SMRT when compared to the wild type receptor.

These data demonstrate that SMRT plays an important role in mediating transcriptional silencing effects of both RAR and TR. These data also suggest that the release of SMRT from receptors could be a prerequisite step in ligand-dependent transactivation by nuclear receptors.

Example 5

Formation of ternary complexes containing SMRT

RAR and TR form heterodimers with RXR, resulting in a complex with high DNA binding ability (Bugge et al., *EMBO J.* **11**:1409-1418 (1992); Yu et al., *Cell* **67**:1251-1266 (1991); and Kliewer et al., *Nature* **355**:446-449 (1992)). Since SMRT interacts with RAR and TR, tests were conducted to determine whether SMRT can also interact with the receptor-DNA complex. Thus, the interaction of SMRT with RXR-RAR heterodimer on a DR5 element (i.e., an AGGTCA direct repeat spaced by five nucleotides) was determined in a gel retardation assay, which is carried out as follows. *In vitro* translated receptor or unprogrammed reticulocyte lysate (URL) was incubated with 1 μg of poly dIdC on ice for 15 minutes in a total volume of 20 μl containing 75 mM KCl, 7.5% glycerol, 20 mM Hepes (pH 7.5), 2 mM DTT and 0.1% NP-40, with or without ligand (in the range of about 10-100 nM employed). A ^{32}P labeled, double stranded oligonucleotide probe was added into the binding reaction (10,000 cpm per reaction), and the reaction was further incubated for 20 minutes at room temperature. The protein-DNA complex was separated on a 5% native polyacrylamide gel at 150 volts.

SMRT is seen to form a ternary complex with the RXR-RAR heterodimer on a DNA reponse element in the gel retardation assay. Addition of ligand releases SMRT from this complex in a dose-dependent manner.

Similarly, SMRT is seen to form a ternary complex with the RXR-TR heterodimer on a TR response element; addition of T3 disrupts the formation of this complex.

These data demonstrate that SMRT can be recruited to DNA response elements via protein-protein interaction with RAR or TR in the absence of hormone. Binding of hormone disrupts receptor-SMRT interaction and releases SMRT from the receptor-DNA complex.

Example 6
Transient transfection assay

CV-1 cells were plated in 24 well plates at a density of 50,000 cells per well. Expression plasmids were transfected into cells by lipofection using DOTAP. In each transfection, 5 ng of GAL-RAR and 15 ng of v-erbA or SMRT were used together with 150 ng of reporter construct containing 4 copies of GAL4 binding sites in front of a minimal thymidine kinase promoter and a CMX-β-gal construct as an internal control. The relative luciferase activity was calculated by normalizing to the β-gal activity.

Example 7
Reversal of transcriptional silencing

Recently, it has been shown that over expression of RAR or TR could reverse the transcriptional silencing effect of the GAL4 DBD fusion of TR (GAL-TR) or RAR (GAL-RAR) (Baniahmad et al., *Mol Cell Biol* **15**:76-86 (1995); and Casanova et al., *Mol Cell Biol* **14**:5756-5765 (1994)), presumably by competition for a limiting amount of a

co-repressor. A similar effect is observed herein when over expression of v-erbA or RAR403 mutants are shown to reverse the silencing effect of GAL-RAR and GAL-TR on the basal activity of a luciferase reporter (see Figure 3A and 3B).

In principle, over expression of SMRT should restore repressor activity when co-expressed with v-erbA or RAR403 competitors. Indeed, results presented in Figure 3C show that both the full length and the C-terminal domain of SMRT (C-SMRT) can titrate out v-erbA or RAR403 competitor activity and re-endow GAL-RAR and GAL-TR with silencing activity. In contrast, neither v-erbA nor SMRT show any effect on the transactivation activity of GAL-VP16 fusion. Thus, SMRT is able to block the titration effect of v-erbA and RAR403 and functionally replaces the putative co-repressor in this system.

Example 8

Direct recruitment of SMRT to a heterologous promoter

If SMRT is the mediator of transcription silencing of TR and RAR by interaction with template-bound unliganded receptors, then direct recruitment of SMRT to a heterologous promoter should result in repression of basal level activity. This was tested by fusing full length SMRT to the GAL4 DBD (GAL-SMRT). The effect of the resulting fusion protein on the activity of the thymidine kinase promoter containing four GAL4 binding sites was analyzed. Figure 3D shows that GAL-SMRT, like GAL-TR, can silence basal promoter activity in a dose-dependent manner. In contrast, GAL-RXR shows no suppression.

These data suggest that SMRT, when recruited to a promoter by direct DNA binding or via association with an unliganded receptor, functions as a potent transcriptional repressor.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: TRANSCRIPTIONAL CO-REPRESSOR THAT INTERACTS WITH NUCLEAR HORMONE RECEPTORS AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 3

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1495 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Glu Ala Trp Asp Ala His Pro Asp Lys Glu Ala Phe Ala Ala Glu
1               5                  10                  15

Ala Gln Lys Leu Pro Gly Asp Pro Pro Cys Trp Thr Ser Gly Leu Pro
            20                  25                  30

Phe Pro Val Pro Pro Arg Glu Val Ile Lys Ala Ser Pro His Ala Pro
        35                  40                  45

Asp Pro Ser Ala Phe Ser Tyr Ala Pro Pro Gly His Pro Leu Pro Leu
    50                  55                  60
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Gly Leu His Asp Thr Ala Arg Pro Val Leu Pro Arg Pro Pro Thr Ile
65                  70                  75                  80

Ser Asn Pro Pro Pro Leu Ile Ser Ser Ala Lys His Pro Ser Val Leu
                85                  90                  95

Glu Arg Gln Ile Gly Ala Ile Ser Gln Gly Met Ser Val Gln Leu His
            100                 105                 110

Val Pro Tyr Ser Glu His Ala Lys Ala Pro Val Gly Pro Val Thr Met
        115                 120                 125

Gly Leu Pro Leu Pro Met Asp Pro Lys Lys Leu Ala Pro Phe Ser Gly
    130                 135                 140

Val Lys Gln Glu Gln Leu Ser Pro Arg Gly Gln Ala Gly Pro Pro Glu
145                 150                 155                 160

Ser Leu Gly Val Pro Thr Ala Gln Glu Ala Ser Val Leu Arg Gly Thr
                165                 170                 175

Ala Leu Gly Ser Val Pro Gly Gly Ser Ile Thr Lys Gly Ile Pro Ser
            180                 185                 190

Thr Arg Val Pro Ser Asp Ser Ala Ile Thr Tyr Arg Gly Ser Ile Thr
        195                 200                 205

His Gly Thr Pro Ala Asp Val Leu Tyr Lys Gly Thr Ile Thr Arg Ile
    210                 215                 220

Ile Gly Glu Asp Ser Pro Ser Arg Leu Asp Arg Gly Arg Glu Asp Ser
225                 230                 235                 240

Leu Pro Lys Gly His Val Ile Tyr Glu Gly Lys Lys Gly His Val Leu
                245                 250                 255

Ser Tyr Glu Gly Gly Met Ser Val Thr Gln Cys Ser Lys Glu Asp Gly
            260                 265                 270

Arg Ser Ser Ser Gly Pro Pro His Glu Thr Ala Ala Pro Lys Arg Thr
        275                 280                 285

Tyr Asp Met Met Glu Gly Arg Val Gly Arg Ala Ile Ser Ser Ala Ser
    290                 295                 300

Ile Glu Gly Leu Met Gly Arg Ala Ile Pro Pro Glu Arg His Ser Pro
305                 310                 315                 320

His His Leu Lys Glu Gln His His Ile Arg Gly Ser Ile Thr Gln Gly
                325                 330                 335

Ile Pro Arg Ser Tyr Val Glu Ala Gln Glu Asp Tyr Leu Arg Arg Glu
            340                 345                 350

Ala Lys Leu Leu Lys Arg Glu Gly Thr Pro Pro Pro Pro Pro Pro Ser
        355                 360                 365

Arg Asp Leu Thr Glu Ala Tyr Lys Thr Gln Ala Leu Gly Pro Leu Lys
    370                 375                 380

Leu Lys Pro Ala His Glu Gly Leu Val Ala Thr Val Lys Glu Ala Gly
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Arg Ser Ile His Glu Ile Pro Arg Glu Glu Leu Arg His Thr Pro Glu
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Leu Pro Leu Ala Pro Arg Pro Leu Lys Glu Gly Ser Ile Thr Gln Gly
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His Asp Val Arg Ser Leu Ile Gly Ser Pro Gly Arg Thr Phe Pro Pro
    450                 455                 460

Val His Pro Leu Asp Val Met Ala Asp Ala Arg Ala Leu Glu Arg Ala
465                 470                 475                 480

Cys Tyr Glu Glu Ser Leu Lys Ser Arg Pro Gly Thr Ala Ser Ser Ser
                485                 490                 495

Gly Gly Ser Ile Ala Arg Gly Ala Pro Val Ile Val Pro Glu Leu Gly
            500                 505                 510

Lys Pro Arg Gln Ser Pro Leu Thr Tyr Glu Asp His Gly Ala Pro Phe
        515                 520                 525

Ala Gly His Leu Pro Arg Gly Ser Pro Val Thr Met Arg Glu Pro Thr
    530                 535                 540

Pro Arg Leu Gln Glu Gly Ser Leu Ser Ser Ser Lys Ala Ser Gln Asp
545                 550                 555                 560

Arg Lys Leu Thr Ser Thr Pro Arg Glu Ile Ala Lys Ser Pro His Ser
                565                 570                 575

Thr Val Pro Glu His His Pro His Pro Ile Ser Pro Tyr Glu His Leu
            580                 585                 590

Leu Arg Gly Val Ser Gly Val Asp Leu Tyr Arg Ser His Ile Pro Leu
        595                 600                 605

Ala Phe Asp Pro Thr Ser Ile Pro Arg Gly Ile Pro Leu Asp Ala Ala
    610                 615                 620

Ala Ala Tyr Tyr Leu Pro Arg His Leu Ala Pro Asn Pro Thr Tyr Pro
625                 630                 635                 640

His Leu Tyr Pro Pro Tyr Leu Ile Arg Gly Tyr Pro Asp Thr Ala Ala
                645                 650                 655

Leu Glu Asn Arg Gln Thr Ile Ile Asn Asp Tyr Ile Thr Ser Gln Gln
            660                 665                 670

Met His His Asn Thr Ala Thr Ala Met Ala Gln Arg Ala Asp Met Leu
        675                 680                 685

Arg Gly Leu Ser Pro Arg Glu Ser Ser Leu Ala Leu Asn Tyr Ala Ala
    690                 695                 700

Gly Pro Arg Gly Ile Ile Asp Leu Ser Gln Val Pro His Leu Pro Val
705                 710                 715                 720

Leu Val Pro Pro Thr Pro Gly Thr Pro Ala Thr Ala Met Asp Arg Leu
                725                 730                 735

Ala Tyr Leu Pro Thr Ala Pro Gln Pro Phe Ser Ser Arg His Ser Ser
            740                 745                 750

Ser Pro Leu Ser Pro Gly Gly Pro Thr His Leu Thr Lys Pro Thr Thr
        755                 760                 765
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Thr Ser Ser Ser Glu Arg Glu Arg Asp Arg Asp Arg Glu Arg Asp Arg
770 775 780

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785 790 795 800

His Ala Pro Ile Trp Arg Pro Gly Thr Glu Gln Ser Ser Gly Ser Ser
805 810 815

Gly Ser Ser Gly Gly Gly Gly Gly Ser Ser Ser Arg Pro Ala Ser His
820 825 830

Ser His Ala His Gln His Ser Pro Ile Ser Pro Arg Thr Gln Asp Ala
835 840 845

Leu Gln Gln Arg Pro Ser Val Leu His Asn Thr Gly Met Lys Gly Ile
850 855 860

Ile Thr Ala Val Glu Pro Ser Lys Pro Thr Val Leu Arg Ser Thr Ser
865 870 875 880

Thr Ser Ser Pro Val Arg Pro Ala Ala Thr Phe Pro Pro Ala Thr His
885 890 895

Cys Pro Leu Gly Gly Thr Leu Asp Gly Val Tyr Pro Thr Leu Met Glu
900 905 910

Pro Val Leu Leu Pro Lys Glu Ala Pro Arg Val Ala Arg Pro Glu Arg
915 920 925

Pro Arg Ala Asp Thr Gly His Ala Phe Leu Ala Lys Pro Pro Ala Arg
930 935 940

Ser Gly Leu Glu Pro Ala Ser Ser Pro Ser Lys Gly Ser Glu Pro Arg
945 950 955 960

Pro Leu Val Pro Pro Val Ser Gly His Ala Thr Ile Ala Arg Thr Pro
965 970 975

Ala Lys Asn Leu Ala Pro His His Ala Ser Pro Asp Pro Pro Ala Pro
980 985 990

Pro Ala Ser Ala Ser Asp Pro His Arg Glu Lys Thr Gln Ser Lys Pro
995 1000 1005

Phe Ser Ile Gln Glu Leu Glu Leu Arg Ser Leu Gly Tyr His Gly Ser
1010 1015 1020

Ser Tyr Ser Pro Glu Gly Val Glu Pro Val Ser Pro Val Ser Ser Pro
1025 1030 1035 1040

Ser Leu Thr His Asp Lys Gly Leu Pro Lys His Leu Glu Glu Leu Asp
1045 1050 1055

Lys Ser His Leu Glu Gly Glu Leu Arg Pro Lys Gln Pro Gly Pro Val
1060 1065 1070

Lys Leu Gly Gly Glu Ala Ala His Leu Pro His Leu Arg Pro Leu Pro
1075 1080 1085

Glu Ser Gln Pro Ser Ser Ser Pro Leu Leu Gln Thr Ala Pro Gly Val
1090 1095 1100

Lys Gly His Gln Arg Val Val Thr Leu Ala Gln His Ile Ser Glu Val
1105 1110 1115 1120

Ile Thr Gln Asp Tyr Thr Arg His His Pro Gln Gln Leu Ser Ala Pro
1125 1130 1135

Leu Pro Ala Pro Leu Tyr Ser Phe Pro Gly Ala Ser Cys Pro Val Leu
1140 1145 1150

Asp Leu Arg Arg Pro Pro Ser Asp Leu Tyr Leu Pro Pro Pro Asp His
1155 1160 1165

Gly Ala Pro Ala Arg Gly Ser Pro His Ser Glu Gly Gly Lys Arg Ser
1170 1175 1180

Pro Glu Pro Asn Lys Thr Ser Val Leu Gly Gly Gly Glu Asp Gly Ile
1185 1190 1195 1200

Glu Pro Val Ser Pro Pro Glu Gly Met Thr Glu Pro Gly His Ser Arg
1205 1210 1215

Ser Ala Val Tyr Pro Leu Leu Tyr Arg Asp Gly Glu Gln Thr Glu Pro
1220 1225 1230

Ser Arg Met Gly Ser Lys Ser Pro Gly Asn Thr Ser Gln Pro Pro Ala
1235 1240 1245

Phe Phe Ser Lys Leu Thr Glu Ser Asn Ser Ala Met Val Lys Ser Lys
1250 1255 1260

Lys Gln Glu Ile Asn Lys Lys Leu Asn Thr His Asn Arg Asn Glu Pro
1265 1270 1275 1280

Glu Tyr Asn Ile Ser Gln Pro Gly Thr Glu Ile Phe Asn Met Pro Ala
1285 1290 1295

Ile Thr Gly Thr Gly Leu Met Thr Tyr Arg Ser Gln Ala Val Gln Glu
1300 1305 1310

His Ala Ser Thr Asn Met Gly Leu Glu Ala Ile Ile Arg Lys Ala Leu
1315 1320 1325

Met Gly Lys Tyr Asp Gln Trp Glu Glu Ser Pro Pro Leu Ser Ala Asn
1330 1335 1340

Ala Phe Asn Pro Leu Asn Ala Ser Ala Ser Leu Pro Ala Ala Met Pro
1345 1350 1355 1360

Ile Thr Ala Ala Asp Gly Arg Ser Asp His Thr Leu Thr Ser Pro Gly
1365 1370 1375

Gly Gly Gly Lys Ala Lys Val Ser Gly Arg Pro Ser Ser Arg Lys Ala
1380 1385 1390

Lys Ser Pro Ala Pro Gly Leu Ala Ser Gly Asp Arg Pro Pro Ser Val
1395 1400 1405

Ser Ser Val His Ser Glu Gly Asp Cys Asn Arg Arg Thr Pro Leu Thr
1410 1415 1420

Asn Arg Val Trp Glu Asp Arg Pro Ser Ser Ala Gly Ser Thr Pro Phe
1425 1430 1435 1440

Pro Tyr Asn Pro Leu Ile Met Arg Leu Gln Ala Gly Tyr Met Ala Ser
1445 1450 1455

Pro Pro Pro Pro Gly Leu Pro Ala Gly Ser Gly Pro Leu Ala Gly Pro

1460 1465 1470

His His Ala Trp Asp Glu Glu Pro Lys Pro Leu Leu Cys Ser Gln Tyr
1475 1480 1485

Glu Thr Leu Ser Asp Ser Glu
1490 1495

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Lys Tyr Asp Gln Trp Glu Glu Ser Pro Pro Leu Ser Ala Asn Ala
1 5 10 15

Phe Asn Pro Leu Asn Ala Ser Ala Ser Leu Pro Ala Ala Met Pro Ile
20 25 30

Thr Ala Ala Asp Gly Arg Ser Asp His Thr Leu Thr Ser Pro
35 40 45

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGGAGGACTG TCCTCCG 17

That which is claimed is:

1. A co-suppressor of steroid/thyroid hormone receptor activity, said co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

2. A co-suppressor according to claim 1 having substantially the same sequence as residues 1-1329 plus 1376-1495, as set forth in SEQ ID NO:1.

3. A co-suppressor according to claim 2 further comprising the amino acid residues set forth in SEQ ID NO:2, i. e., residues 1330-1375 of SEQ ID NO:1.

4. An antibody raised against the co-suppressor of claim 1.

5. A method to block the repressing effect of co-suppressors having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising administering an effective amount of an antibody according to claim 4.

6. Isolated polynucleic acid encoding the co-suppressor of claim 1.

7. A vector containing polynucleic acid acording to claim 6.

8. A complex comprising the co-suppressor of claim 1 and a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors, wherein said member contains a silencing domain which represses basal level promoter activity of target genes.

9. A complex according to claim 8 wherein said homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors is selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, retinoic acid receptor-retinoid X receptor heterodimer or retinoid X receptor homodimer.

10. A complex according to claim 8 further comprising a response element for said member of the steroid/thyroid hormone superfamily of receptors.

11. A method to dissociate the complex of claim 8, said method comprising contacting said complex with ligand for said member of the steroid/thyroid hormone superfamily of receptors.

12. A method to repress the activity of a member of the steroid/thyroid hormone superfamily of receptors containing a silencing domain which represses basal level promoter activity of target genes, said method comprising contacting said member of the steroid/thyroid hormone superfamily of receptors with a sufficient quantity of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors so as to repress the activity of said member.

13. A method according to claim 12 wherein said member of the steroid/thyroid hormone superfamily of receptors is selected from thyroid hormone receptor, retinoic acid receptor, vitamin D receptor or peroxisome proliferator activated receptor.

14. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising comparing the size of the complex of claim 8 upon exposure to test compound, relative to the size of said complex in the absence of test compound.

15. A method according to claim 14 wherein said complex further comprises a response element for said member of the steroid/thyroid hormone superfamily of receptors.

16. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, without substantially activating said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of

receptors, wherein said response element is operatively linked to a reporter, and

optionally, the co-suppressor of claim 1, and

wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

optionally, the co-suppressor of claim 1, and thereafter

selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, but

substantially lacking the ability to activate steroid/thyroid hormone receptor activity.

17. A method according to claim 16 wherein said mutant receptor is selected from RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers or TR160-containing heterodimers.

18. A method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

optionally, the co-suppressor of claim 1, and

wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

optionally, the co-suppressor of claim 1, and thereafter

selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of compound, and

substantially the same reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

19. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activate said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

optionally, the co-suppressor of claim 1, and

wherein said second expression system comprises a complex comprising:

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

the same response element-reporter combination as employed in said first expression system, and

optionally, the co-suppressor of claim 1, and thereafter

selecting those compounds which provide:

increased reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound, and

substantially increased reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor.

20. A modified form of the co-suppressor of claim 1 selected from:

full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 DNA binding domain,

full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 activation domain, or

full length silencing mediator for retinoic acid and thyroid receptors plus glutathione S-transferase (GST) tag.

21. A modified co-suppressor according to claim 20, wherein said modified co-suppressor comprises full length silencing mediator for retinoic acid and thyroid receptors plus GAL4 activation domain.

22. A method to identify compounds which disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with steroid/thyroid hormone receptors, without substantially activating said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor according to claim 21,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

said modified co-suppressor,

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but

has lost its ability to repress basal level promoter activity of target genes, and

the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

a lower reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of disrupting the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with steroid/thyroid hormone receptors, without substantially activating said receptor.

23. A method according to claim 22 wherein said mutant receptor is selected from RAR403 homodimers, RAR403-containing heterodimers, TR160 homodimers or TR160-containing heterodimers.

24. A method according to claim 22, wherein the host is a mammalian or yeast cell.

25. A method to identify compounds which activate steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor according to claim 21,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises:

said modified co-suppressor,

a homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but

has lost its ability to repress basal level promoter activity of target genes, and

the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

a higher reporter signal upon exposure of said compound to said second expression system, relative to reporter signal in the absence of compound, and

substantially the same reporter signal upon exposure of said compound to said first expression system, relative to reporter signal in the absence of compound,

wherein said selected comounds are capable of activating steroid/thyroid hormone receptor activity, but substantially lack the ability to disrupt the complex of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors and a steroid/thyroid hormone receptor.

26. A method according to claim 25, wherein the host is a mammalian or yeast cell.

27. A method to identify compounds which activate a steroid/thyroid hormone receptor, and disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with said receptor, said method comprising:

comparing the reporter signal produced by two different expression systems in the absence and presence of test compound,

wherein said first expression system comprises a complex comprising:

a modified co-suppressor according to claim 21,

a homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer or retinoic acid receptor-retinoid X receptor heterodimer, and

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a reporter, and

wherein said second expression system comprises a complex comprising:

said modified co-suppressor,

the same homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors as employed in said first expression system, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes, and

the same response element-reporter combination as employed in said first expression system, and thereafter

selecting those compounds which provide:

a reduction in reporter signal upon exposure of compound to said first expression system, relative to reporter signal in the absence of said compound, and

increased reporter signal upon exposure of compound to said second expression system, relative to reporter signal in the absence of said compound,

wherein said selected compounds are capable of activating a steroid/thyroid hormone receptor and disrupting a complex comprising steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

28. A method according to claim 27, wherein the host is a mammalian or yeast cell.

29. A method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with said receptor, said method comprising:

comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

wherein said combination expression system comprises:

a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a second homodimeric or heterodimeric form of the same member of the

steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but has lost its ability to repress basal level promoter activity of target genes,

wherein either said first homodimer (or heterodimer) or said second homodimer (or heterodimer) is operatively linked to a GAL4 DNA binding domain,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a first reporter,

a GAL4 response element, wherein said response element is operatively linked to a second reporter, and thereafter

identifying as capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, but substantially lacking the ability to activate steroid/thyroid hormone receptor activity those compounds which provide:

a higher reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member,

relative to reporter signal in the absence of said compound, or

identifying as capable of activating steroid/thyroid hormone receptor activity, but substantially lacking the ability to relieve the suppression caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and

substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

identifying as capable of relieving the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

increased reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, and

substantially increased reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound.

30. A method according to claim 29 wherein said combination expression system further comprises a co-suppressor of steroid/thyroid hormone receptor activity, said co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors.

31. A method to identify compounds which activate a steroid/thyroid hormone receptor and/or disrupt the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with said receptor, said method comprising:

comparing the reporter signals produced by a combination expression system in the absence and presence of test compound,

wherein said combination expression system comprises:

a modified co-suppressor according to claim 21,

a first homodimeric or heterodimeric member of the steroid/thyroid hormone superfamily of receptors selected from thyroid hormone receptor homodimer, thyroid hormone receptor-retinoid X receptor heterodimer, retinoic acid receptor homodimer, or retinoic acid receptor-retinoid X receptor heterodimer,

a second homodimeric or heterodimeric form of the same member of the steroid/thyroid hormone superfamily of receptors as employed in said first homodimer or heterodimer, wherein said member is mutated such that it retains hormone dependent activation activity but

has lost its ability to repress basal level promoter activity of target genes,

wherein either said first homodimer (or heterodimer) or said second homodimer (or heterodimer) is operatively linked to a GAL4 DNA binding domain,

a response element for said member of the steroid/thyroid hormone superfamily of receptors, wherein said response element is operatively linked to a first reporter,

a GAL4 response element, wherein said response element is operatively linked to a second reporter, and thereafter

identifying as capable of disrupting the ability of a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors to complex with steroid/thyroid hormone receptor, without substantially activating steroid/thyroid hormone receptor, those compounds which provide:

a lower reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

substantially the same reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound, or

identifying as capable of activating steroid/thyroid hormone receptor activity, but

substantially lacking the ability to disrupt a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors those compounds which provide:

a higher reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of compound, and

substantially the same reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, or

identifying as capable of disrupting a complex comprising a steroid/thyroid hormone receptor and a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, and activating said receptor those compounds which provide:

a reduction in reporter signal from the reporter responsive to the first member upon exposure of said compound to said first member, relative to reporter signal in the absence of said compound, and

increased reporter signal from the reporter responsive to the second member upon exposure of said compound to said second member, relative to reporter signal in the absence of said compound.

32. A method to identify compounds which relieve the suppression of steroid/thyroid hormone receptor activity caused by a co-suppressor having a structure and function characteristic of the silencing mediator for retinoic acid and thyroid receptors, said method comprising determining the effect of adding test compound to an expression system comprising:

a modified member of the steroid/thyroid hormone superfamily of receptors, wherein said modified member contains an activation domain which renders said receptor constitutively active,

a fusion protein comprising the receptor interaction domain of SMRT operatively linked to the GAL4 DNA binding domain, and

a GAL4 response element operatively linked to a reporter.

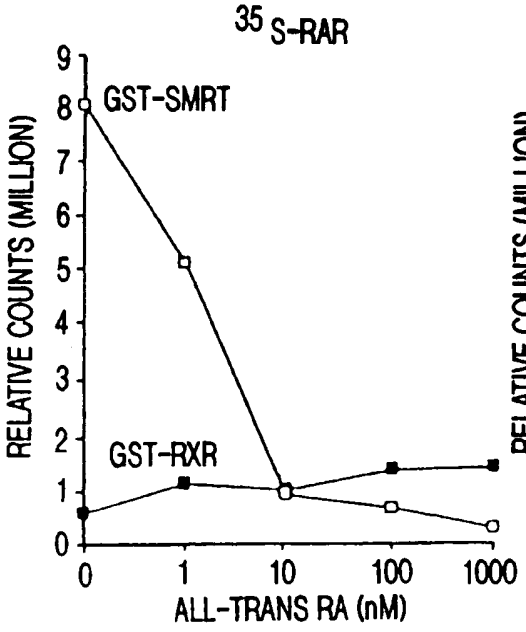
35 S-RAR
GST-SMRT
GST-RXR
RELATIVE COUNTS (MILLION)
0
1
2
3
4
5
6
7
8
9
0
1
10
100
1000
ALL-TRANS RA (nM)

FIG. 1A

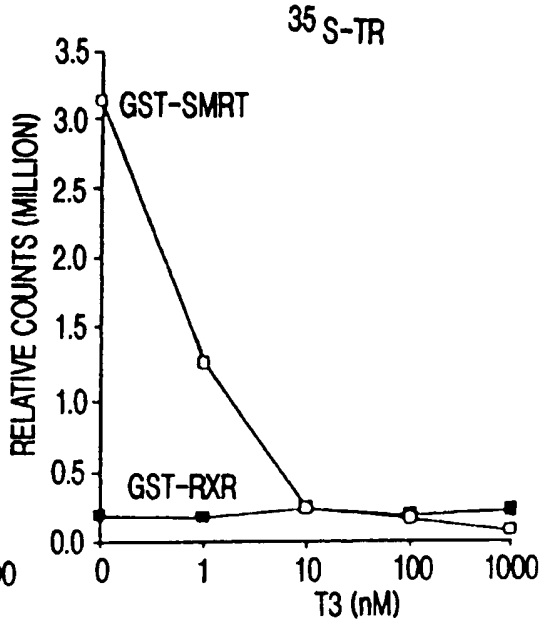
35 S-TR
GST-SMRT
GST-RXR
RELATIVE COUNTS (MILLION)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
1
10
100
1000
T3 (nM)

FIG. 1B

```
   1 MEAWDAIPDKEAFAAEAQKLPGDPPCWTSGLPFPVPPREVIKASPHAPDP
  51 SAFSYAPPGHPLPLGLHDTARPVLPRPPTISNPPPLISSAKHPSVLERQI
 101 GAISQGMSVQLHVPYSEHAKAPVGPVTMGLPLPHDPKKLAPFSGVKQEQL
 151 SPRGQAGPPESLGVPTAQEASVLRGTALGSVPGGSITKGIPSTRVPSDSA
 201 ITYRGSITHGTPADVLYKGTITRIIGEDSPSRLDRGREDSLPKGHVIYEG
 251 KKGHVLSYEGGMSVTQCSKEDGRSSSGPPHETAAPKRTYDHMEGRVGRAI
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 351 REAKLLKREGTPPPPPPSRDLTEAYKTQALGPLKLKPAHEGLVATVKEAG
 401 RSIHEIPREELRHTPELPLAPRPLKEGSITQGTPLKYDTGASTTGSKKHD
 451 VRSLIGSPGRTFPPVHPLDVMADARALERACYEESLKSRPGTASSSGGSI
 501 ARGAPVIVPELGKPRQSPLTYEDHGAPFAGHLPRGSPVTMREPTPRLQEG
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                                  ┌──→ C-SMRT
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1399 LA..SGDRPPSVSSVHSEGDCNRRTPLTNRVWEDRPSSAGSTPFPYNPLI

1447 NRLQAGVMASPPPPGLPAGSGPL..AGPHHA..WDEEPKPLLCSQYETL

1492 SDSE*  1495
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FIG. 2

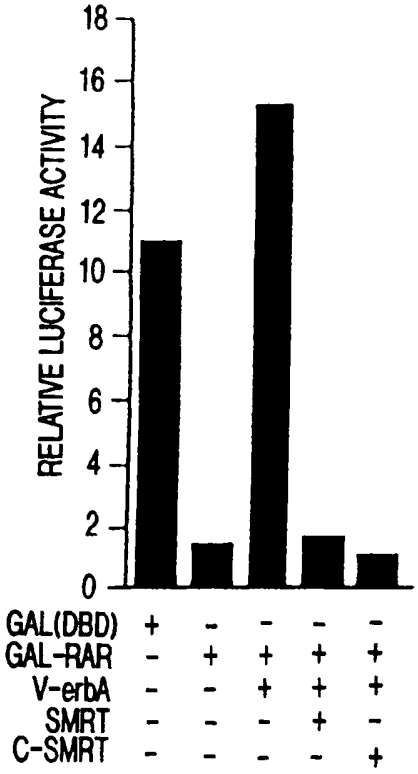

FIG. 3A

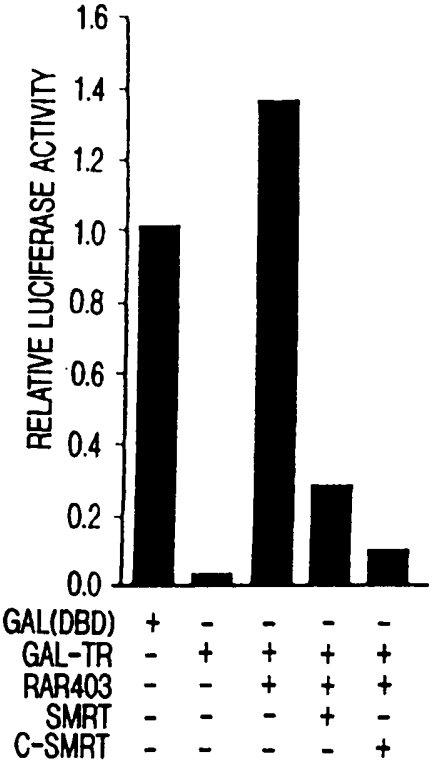

FIG. 3B

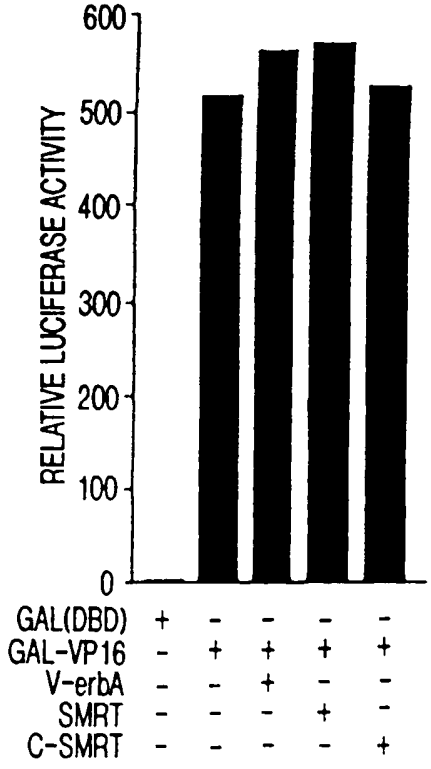

FIG. 3C

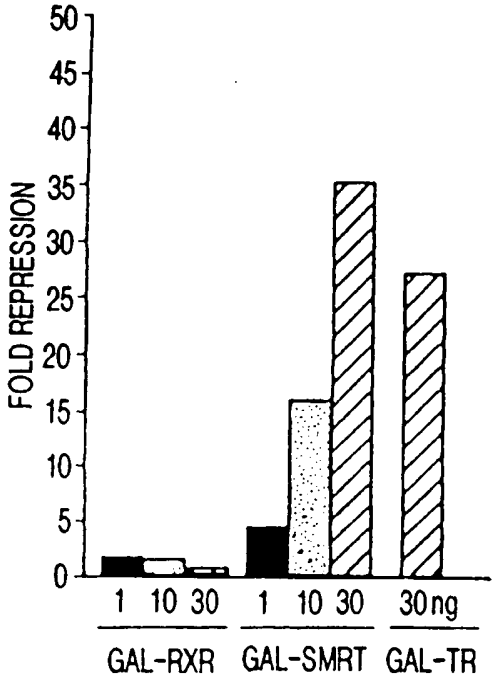

FIG. 3D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12371

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.21, 69.1, 69.7, 240.1, 252.3, 320.1; 530/350, 300, 324, 389.2; 536/23.5, 23.1; 436/501

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sucov et al. Retinoic acid and retinoic acid receptors in development. Molec. Neurobiol. 19 July 1995, Vol. 10, Nos. 2/3, pages 169-184, especially pages 171-181.	1-3, 6-32
Y	Leid et al. Multiplicity generates diversity in the retinoic acid signalling pathways. Trends Biochem. Sci. October 1992, Vol. 17, pages 427-433, especially pages 427-432.	1-3, 6-32
X --- Y	US 5,283,173 A (S. FIELDS) 01 February 1994, column 3-4.	1 ----- 2-3, 6-32
X ---- Y	US 5,317, 090 A (H.B. DE THE) 31 May 1994, columns 5-6.	1 ----- 2-3, 6-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 AUGUST 1996	Date of mailing of the international search report 13 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KENNETH A SORENSEN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12371

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 1/20, 15/00, 5/00; G01N 33/53, 33/567, 33/566; C12P 21/06, 21/04; C07K 16/00, 1/00; A61K 39/395, 38/00; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.1, 7.21, 69.1, 69.7, 240.1, 252.3, 320.1; 530/350, 300, 324, 389.2; 536/23.5, 23.1; 436/501

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAPLUS, MEDLINE, EMBASE, BIOSIS, JICST-EPLUS, WPIDS, PATOSEP, CONFSCI, DISSABS search terms: steroid hormone receptor, activity, suppressor, co-suppressor, binding, complex, heterodimer, homodimer, screen, assay, method

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-3 and 6-32, drawn to a co-suppressor, polynucleic acid, vector, complex comprising co-suppressor and methods of screening using co-suppressor.

Group II, claim(s) 4-5, drawn to an antibody and a method of administering said antibody.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of group I share the special technical feature of a co-suppressor of steroid/thyroid hormone receptor activity whereas claims of group II do not share this special technical feature but instead share the special technical feature of an antibody and in addition each group have materially different chemical structures and materially different functional properties. These chemical structures and functional properties are the special technical features that identify each invention and distinguish each from the other because none of the special technical features is shared by the separate groups. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.